ISOLATION, GENE EXPRESSION AND STRUCTURAL ANALYSIS OF FRUIT BROMELAIN FROM *Ananas comosus* CULTIVAR MD 2

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MASTER OF SCIENCE

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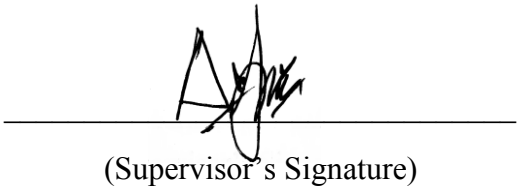
I hereby declare that I have checked this thesis and in my opinion, this thesis is adequate in terms of scope and quality for the award of the degree of Master of Science.



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I hereby declare that the work in this thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at Universiti Malaysia Pahang or any other institutions.

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ABSTRAK

Ananas comosus atau nanas adalah salah satu buah tropika yang popular di dunia. Terdapat pelbagai kultivar nanas dengan ciri-ciri yang berbeza. *A. comosus* kultivar MD 2 adalah hibrida nanas yang paling berjaya dengan warna emasnya yang menarik, rasa manis dan kualiti terunggul. Nanas kaya dengan enzim proteolitik seperti bromelain buah. Dalam kajian ini, bromelain buah telah dicirikan dan dibandingkan di antara *A. comosus* kultivar MD 2 yang ranum dan muda. Selain itu, transkrip bromelain buah juga diperolehi melalui teknik "conventional Polymerase Chain Reaction" dan pengklonan. Maklumat jujukan gen bromelain buah telah digunakan untuk pemodelan 3D struktur dan diikuti dengan analisis termostabiliti. Untuk mengaji perbezaan tahap ekspresi gen dan aktiviti proteolitik buah bromelain antara *A. comosus* kultivar MD 2 yang ranum dan muda, "quantitative Polymerase Chain Reaction" dan ujian enzim kasein telah dilaksanakan. Maklumat jujukan gen bromelain buah telah dianalisis menggunakan beberapa program bioinformatik seperti "BioEdit", "BLAST", "InterProScan" dan "ProtParam". 3D struktur bromelain buah telah dimodelkan menggunakan "MODELLER" dan simulasi molekul dinamik. Selanjutnya, termostabiliti bromelain buah juga telah dinilai menggunakan ujian enzim kasein dan simulasi molekul dinamik. Hasil kajian menunjukkan bahawa pengekspresan bromelain buah telah diturunkan sebanyak 90 % dalam *A. comosus* kultivar MD 2 yang ranum. Ia juga didapati bahawa *A. comosus* kultivar MD 2 yang muda mempunyai aktiviti yang lebih tinggi (1.91 ± 0.08 U/mL) berbanding dengan *A. comosus* kultivar MD 2 yang ranum (1.13 ± 0.09 U/mL). Di samping itu, tiga jujukan bromelain buah iaitu OAY62650.1, OAY68270.1 dan OAY85858.1 telah dipilih untuk diklon. Keputusan penjujukan menunjukkan perubahan nukleotida. Selain itu, "Verify 3D", "ERRAT" dan "PROCHECK" menunjukkan model bromelain buah yang dihasilkan mempunyai kualiti yang tinggi. Analisis struktur mengungkapkan interaksi antara "pro-domain" dan "catalytic domain". "Binding subsites" bromelain buah termasuk His160, Trp183, Glu19 dan Asn159 juga telah dikenal pasti. Tambahan pula, profil haba bromelain buah telah dibina. Suhu optimum bromelain buah telah dikenal pasti pada 60 °C. Apabila suhu meningkat, kestabilan bromelain buah telah terganggu. Hasil simulasi menunjukkan bahawa pemusnahan struktur bromelain buah mungkin disebabkan gelung sekunder yang fleksibel serta asid amino tanpa cas dan hidrofilik. Maklumat yang diperoleh daripada kajian ini dijangka dapat menambahbaik penggunaan bromelain buah pada masa akan datang.

ABSTRACT

Ananas comosus, or commonly known as pineapple, is one of the most popular tropical fruits in the world. There are various pineapples cultivars with distinct characteristics. *A. comosus* cultivar MD 2 is the most successful pineapple hybrid with its attractive gold colour, super sweet taste and superior quality. Pineapple is rich in proteolytic enzymes such as fruit bromelain. In this study, fruit bromelain was characterised and compared between ripe and unripe *A. comosus* cultivar MD 2. Besides that, fruit bromelain transcripts were also isolated by conventional PCR and cloning. The extracted fruit bromelain sequence information was used in tertiary structure modelling followed by thermostability analysis. To investigate the gene expression level and proteolytic activity of fruit bromelain of *A. comosus* cultivar MD 2, quantitative Polymerase Chain Reaction (PCR) and casein enzymatic assay were conducted. Meanwhile, the sequence information of fruit bromelain was analysed using several bioinformatic tools including BioEdit, BLAST, InterProScan and ProtParam. The tertiary structure of the selected fruit bromelain sequences was modelled using MODELLER and refined via molecular dynamics simulation. Subsequently, the thermostability of fruit bromelain was evaluated using casein enzymatic assay and molecular dynamics simulation. The result revealed that fruit bromelain was down-regulated by 90 % in ripe *A. comosus* cultivar MD 2. It was also found that unripe *A. comosus* cultivar MD 2 has higher fruit bromelain activity (1.91 ± 0.08 U/mL) than ripe *A. comosus* cultivar MD 2 (1.13 ± 0.09 U/mL). On the other hand, three fruit bromelain sequences namely OAY62650.1, OAY68270.1 and OAY85858.1 were selected as sequences of interest to be isolated and cloned. The sequencing result demonstrated several nucleotides alteration in the isolated sequences. Moreover, Verify 3D, ERRAT and PROCHECK showed that the generated fruit bromelain models have high stereochemical quality. Structural analysis revealed interactions between the pro-domain and catalytic domain. Binding subsites of fruit bromelain His160, S2 Trp183, Glu19 and Asn159 were also identified. The His160, Trp183 and Glu19 were found conserved between fruit bromelain and papain. In addition, the thermal profile of MD 2 fruit bromelain was constructed. The optimum temperature of fruit bromelain was identified at 60 °C which is within the expected range. As the temperature raises, the stability of fruit bromelain was disrupted. The simulation result showed that fruit bromelain structures destruction may be due to flexible secondary loops as well as the presence of non-charged and hydrophilic amino acids residues. The information obtained from this study is expected to improve the applications of fruit bromelain in the future.

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LIST OF SYMBOLS

%	Percent
α	Alpha
Å	Angstrom
β	Beta
°C	Degree celcius
R^2	Coefficient of determination

LIST OF ABBREVIATIONS

3D	Three-dimensional
A	Absorbance
Ala	Alanine
Arg	Arginine
Asp	Aspartate
Asn	Asparagine
BLAST	Basic Local Alignment Tool
bp	Base pair
cDNA	Complementary deoxyribonucleic acid
C_q	Quantification cycle
Cys	Cysteine
dNTP	Deoxynucleotide
DOPE	Discrete optimized protein energy
EC	Enzyme Commission
ETP	Economic Transformation Programme
FAO	Food and Agriculture Organization of the United Nations
F-C	Folin Ciocalteu
fs	Femtosecond
g	gram
GC	Guanine cytosine
Gly	Glycine
Gln	Glutamine
Glu	Glutamate
GROMACS	GROningen MAchine for Chemical Simulations
His	Histidine
I29	Cathepsin pro-peptide inhibitor
Ile	Isoleucine
LB	Luria Bertani
Leu	Leucine
kDa	Kilodalton
kg	Kilogram

LINCS	LINear Constraint Solver
Lys	Lysine
Met	Methionine
min	Minute
miRNA	Micro Ribonucleic Acid
mg	Milligram
mL	Millilitre
MPIB	Malaysian Pineapple Industrial Board
mRNA	Messsenger Ribonucleic Acid
N. A	Not available
NCBI	National Center for Biotechnology Information
ng	Nanogram
NKEA	National Key Economic Area
nm	Nanometer
NMR	Nuclear Magnetic Resonance
NPT	Constant number of particles, volume and temperature
NVT	Constant number of particles, pressure and temperature
ns	Nanosecond
PCR	Polymerase Chain Reaction
PDB	Protein Database Bank
Phe	Phenylalanine
pI	Isoelectric point
PLCE	Papain-like cysteine endopeptidase
PME	Particle mesh Ewald method
pNA	p-nitroalanine
Pro	Proline
ps	Picosecond
qPCR	Quantitative polymerase chain reaction
Rg	Radius of gyration
RMSD	root-mean-square deviation
RMSF	root-mean-square fluctuation
RNA	Ribonucleic acid
rpm	Revolutions per minute

rRNA	Ribosomal ribonucleic acid
RT-PCR	Reverse transcription polymerase chain reaction
SASA	Solvent accessible surface area
SAVES	Structure Analysis and Verification Server
SD	Standard deviation
SE	Standard error
Sec	Second
Ser	Serine
snRNA	Small nuclear ribonucleic acid
SPDBV	Swiss PDB Viewer
TA	Thymine adenine
Thr	Threonine
Tm	Melting temperature
Trp	Tryptophan
tsRNA	tRNA-derived small ribonucleic acid
Tyr	Tyrosine
µL	Microliter
µM	Micrometer
µmol	Micromoles
Val	Valine

LIST OF APPENDICES

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